Clontech Laboratories, Inc.

HisTALON™ Gravity Column Purification Kit User Manual

Cat. Nos. 635651, 635654 & 635655

PT4435-1 (032216)

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: tech@clontech.com

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.543.6116

Table of Contents

Table of Figures

Table of Tables

Contact Us	
Customer Service/Ordering	**Technical Support**
tel: 800.662.2566 (toll-free)	tel: 800.662.2566 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 650.424.1064
web: www.clontech.com	web: www.clontech.com
e-mail: orders@clontech.com	e-mail: tech@clontech.com

I. Introduction

Clontech's **HisTALON Gravity Columns** are ready-to-use columns for the efficient purification of his-tagged proteins, using gravity-flow-based protocols, from bacterial, mammalian, and baculovirus-infected cells. The columns are prepacked with our TALON® Resin. TALON is a tetradentate chelator charged with cobalt, and is specific for his-tagged proteins (Chaga *et al.*, 1994; Froelich *et al.*, 1996; Hochuli *et al.*, 1987 & 1988; Porath *et al.*, 1975; Stephens *et al.*, 1997). The stable chelation of the Co^{2+} ion, combined with the specificity of the TALON reactive core, deliver unmatched purity. More than 20 mg of his-tagged AcGFP1 can be adsorbed on one HisTALON Column.

These columns enable fast, easy, and reproducible chromatographic separation—and can be regenerated for multiple uses. However, we recommend that you reuse a column only to purify different batches of the same protein. If you plan to purify multiple proteins using the same column, you must utilize the "Complete Regeneration" method described in the TALON Metal Affinity Resins User Manual (PT1320-1) at **www.clontech.com/manuals**

Figure 1. HisTALON Gravity Columns provide highly reproducible and rapid his-tagged protein purification.

II. List of Components

Store all components at 4ºC.

HisTALON Gravity Columns (Cat. No. 635655)

- 5 HisTALON Gravity Columns (1 ml each)

HisTALON Buffer Set (Cat. No. 635651)

- 2 x 250 ml HisTALON Equilibration Buffer
- 1 x 200 ml HisTALON Elution Buffer
- 1 x 100 ml HisTALON xTractor™ Buffer

HisTALON Gravity Column Purification Kit (Cat. No. 635654)

- 1 HisTALON Gravity Columns (5 x 1 ml) (Cat. No. 635655)
- 1 HisTALON Buffer Set (Cat. No. 635651)

III. Additional Materials Needed

A. Buffers

Extraction, equilibration, and elution buffers, sufficient for up to 20 purification runs are supplied in the HisTALON Gravity Column Purification Kit (Cat. No. 635654) and the HisTALON Buffer Set (Cat. No. 635651)—see Section II.

B. Enzymes

- **Benzonase** (Sigma, Cat. No. E8263-5KU)
- **Recombinant DNase I** (Takara, Cat. No. 2270A)

C. Optional

- **PD-10 desalting columns** (GE Healthcare, Cat. No. 17-0851-01) to remove incompatible reagents (Section IV) from starting samples prior to loading onto the columns, or to remove excess imidazole from the final sample when required for downstream applications.

IV. General Considerations

Use of HisTALON Gravity Columns

Please note the following recommendations when using HisTALON Gravity Columns:

- For elution, use the Elution Buffer supplied in the HisTALON Gravity Column Purification Kit (Cat. No. 635654) and the HisTALON Buffer Set (Cat. No. 635651), or a highly pure, low-absorbance imidazole (Section III.A).
- Do not use buffers containing EDTA, chelator-containing protease inhibitors or other additives, or strong reducing agents such as DTT with the columns. If any of these reagents are present in the sample, it must be desalted on a PD-10 column (Section III.E) before loading it onto the column.

V. Sample Preparation & Purification

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING.

Use this procedure to (A) prepare your his-tagged protein sample for (B) purification using His TALON Gravity Columns.

A. PROTOCOL: Sample Preparation

Use this method to prepare your protein sample for gravity-flow-based purification:

1. Add 2 ml of HisTALON xTractor Buffer per 100 mg of cell pellet. Gently pipet up and down until the cell pellet is fully resuspended. We recommend using between 200 mg and 1 g of pellet from *E. coli*- or baculovirus-infected cells expressing the target protein.

   NOTE: HisTALON xTractor Buffer is supplied in the HisTALON Gravity Column Purification Kit (Cat. No. 635654) and the HisTALON Buffer Set (Cat. No. 635651), or sold separately (Cat. Nos. 635623, 635625 & 635656).

2. To the resuspended pellet, add 1 µl of Benzonase or DNAse I (Section III.B) for every 2 ml of HisTALON xTractor Buffer used (i.e., every 100 mg of cell pellet), and mix gently.
3. Incubate on ice, with intermittent mixing, for 15 min. Centrifuge for 20 min at 10,000 x g at 4°C.
4. Carefully collect the clear supernatant—this is your starting sample.

B. PROTOCOL: Gravity-Flow Column Purification

1. Equilibrate the column and all buffers to the working temperature. Before opening the column, fully suspend the matrix (to prevent loss of resin that may have settled near the top cap). Perform purifications at room temperature or at 4°C.
2. Degas all solutions.
3. Equilibrate the column with 5–10 column volumes of the Equilibration Buffer.
4. For maximum extraction and binding, prepare the sample using our HisTALON xTractor Buffer (Section V.A). If you used incompatible reagents (Section IV) during the extraction, desalt the sample on a PD-10 column (Section III.E) before proceeding to Step 7.
5. Load the clarified sample onto the column and collect 1 ml fractions.
6. Wash the column with 8 column volumes of Equilibration Buffer followed by 7 column volumes of Wash Buffer (i.e., Equilibration Buffer containing 10 mM imidazole). See Section III.A for instructions on preparing Wash Buffer.

   NOTE: If you are using the buffers supplied in the HisTALON Buffer Set (Cat. No. 635651) or the HisTALON Gravity Column Purification Kit (Cat. No. 635654), prepare the Wash Buffer by mixing 660 µl of Elution Buffer with 9.34 ml of Equilibration Buffer.
7. Elute with approximately 5–8 column volumes of Elution Buffer (containing 150 mM imidazole) and collect 1 ml fractions. Monitor protein elution by measuring the absorbance of the fractions at 280 nm or performing a Bradford assay (Bradford, 1976). The collected fractions can be analyzed by SDS-PAGE.
8. If necessary for downstream applications, remove excess imidazole by gel filtration on a PD-10 column (Section III.E).
9. The HisTALON Gravity Column can be regenerated quickly by adding 20 ml of Equilibration Buffer or by washing with 10 ml of 20 mM MES, 0.3 M NaCl pH 5.0 buffer. Regeneration allows the column to be reused to purify the same protein multiple times without significant loss of binding capacity.
10. For extended storage (over 1 week), wash the column with five column volumes of water after each use and store in 20% ethanol. Attach supplied bottom cap, followed by the top plug. Store the column at 4°C.

VI. Troubleshooting Guide

Table 1. Troubleshooting Guide for His60 Ni Gravity Columns & Resin

Description of Problem	Possible Explanation	Solution
Low target yield	Poor expression of target protein	Optimize bacterial expression conditions.
	Target protein forms inclusion bodies	• Decrease temperature to 25°C or lower during induction to minimize inclusion body formation. • Solubilize inclusion bodies and perform the purification in the presence of 8 M urea or 6 M guanidinium HCl.
	Inefficient target extraction	Use our xTractor Buffer.
	Inaccessible polyhistidine tag	Purify in presence of 8 M urea or 6 M guanidinium HCl.
Impurities in eluate	Insufficient washing	Increase wash volume or add intermediate wash at 20 mM imidazole. (This can result in partial loss of target protein.)
Low flow rate	Clogged column	Apply only clarified extract, and decrease the amount of loaded sample.
	Viscous sample	Treat sample with Benzonase or DNase I, as described in Section V.A.

VII. References

Bradford, M. M. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254.

Chaga, G., Widersten, M., Andersson, L., Porath, J., Danielson, U. H. & Mannervik, B. (1994) Engineering of a metal coordinating site into human glutathione transferase M1-1 based on immobilized metal ion affinity chromatography of homologous rat enzymes. *Protein Eng.* **7**(9):1115–1119.

Froelich, C. J., Turbov, J. & Hanna, W. (1996) Human perforin: rapid enrichment by immobilized metal affinity chromatography (IMAC) for whole cell cytotoxicity assays. *Biochem. Biophys. Res. Commun.* **229**(1):44–49.

Hochuli, E., Döbeli, H. & Schacher, A. (1987) New metal chelate adsorbent selective for proteins and peptides containing neighboring histidine residues. *J. Chromatogr.* **411**:177–184.

Hochuli, E., Bannwarth, W., Döbeli, H., Gentz, R. & Stüber, D. (1988) Genetic approach to facilitate purification of novel recombinant proteins with a novel metal chelate adsorbent. *Bio/Technology* **6:**1321–1325.

Porath, J., Carlsson, J., Olsson, I. & Belfrage, G. (1975) Metal chelate affinity chromatography, a new approach to protein fractionation. *Nature* **258**:598–599.

Stephens, L. R., Eguinoa, A., Erdjument-Bromage, H., Lui, M., Cooke, F., Coadwell, J., Smrcka, A. S., Thelen, M., Cadwallader, K., Tempst, P. & Hawkins, P. T. (1997) The G beta gamma sensitivity of a PI3K is dependent upon a tightly associated adaptor, p101. *Cell* 89(1):105–114.

Notice to Purchaser

Our products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Clontech Laboratories, Inc.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at http://www.clontech.com. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.

Clontech®, the Clontech logo, HisTALON, ProteoGuard, TALON®, and xTractor are trademarks of Clontech Laboratories, Inc. All other trademarks are the property of their respective owners. Certain trademarks may not be registered in all jurisdictions. Clontech Laboratories, Inc. is a Takara Bio Company. ©2016 Clontech Laboratories, Inc.

This document has been reviewed and approved by the Clontech Quality Assurance Department.